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## Profile

# Kits to Dye For: A Profile of Sequencing Kits for Automated DNA Sequencers

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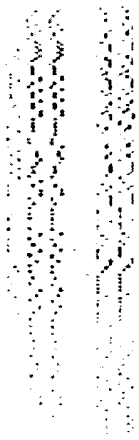
Chart 1

In the long series of events inherent in automated DNA sequencing, cranking out DNA labeled with fluorescent tags is, of course, the most important element of a successful procedure. Without properly labeled sequence ladders to analyze, those expensive, automated DNA sequencers have little to do. So to keep them busy, LabConsumer checked out fluorescent automated DNA sequencing kits from eight manufacturers.



The kits profiled exploit two methods for labeling sequencing products: the incorporation of fluorescent dye-labeled primers and the incorporation of dye-labeled dideoxynucleotides (ddNTPs), or dye terminators. The use of dye-labeled primers is the most common method because the modified forms of Taq and the other novel polymerases used in these kits discriminate against dye-labeled ddNTPs and are unable to efficiently incorporate them into the reaction products. Expressly engineered polymerases, on the other hand, such as ABI's AmpliTaq DNA Polymerase, FS and Amersham's Thermo Sequenase, have been developed to incorporate dye-labeled ddNTP's more efficiently. These engineered enzymes have a decreased discrimination function against ddNTP incorporation that not only makes the use of dye-terminators possible, but also improves the dye-primer chemistry as well, resulting in even signal intensities for both.

Opinions differ about which methodology is best. Fluorescent-labeled ddNTP procedures are probably the least expensive, especially if several primers are needed to sequence a particular template, but this use requires knowledge of the downstream sequence to design those primers. In contrast, the protocols for fluorescent-labeled primers are reportedly faster and touted to produce more accurate and reproducible results. However, these methods are frequently limited by the range of available fluorescent primers and the expense required to synthesize them.

Most of the kits belong to the cycle sequencing category and the remainder use classical, isothermic incubations. A few, such as the SequiTherm kits from Epicentre Technologies and the IR Taq DNA Sequencing Kit from Boehringer Mannheim, function well in both modes. Regardless of the mode, however, the number of bases read past the primer under optimal conditions, as reported by some manufacturers, now exceeds 1,000. PE Applied Biosystems reports a survey stating that te average read length in the United States is about 500 bases, which is probably a better indication of the actual read lengths produced considering the various labs and skill levels involved.